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Experimental and Clinical Studies on Barrett's Esophagus

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The etiology and pathogenesis of the columnar epithelium-lined lower esophagus have been much discussed since 1950 when BARRETT[4] first described the condition as congenital short esophagus with an intrathoracic stomach. By the current accepted concept, Barrett's esophagus is believed to be an acquired disorder in which the normal squamous lining is damaged by acid peptic gastroesophageal reflux and is replaced by columnar epithelium.[2,11,21,23,57,61] Experimentally, BREMNER et al[11] and WONG et al[61] showed that columnar mucosa could migrate upward into the esophagus after loss of the squamous lining. In addition, HAMILTON[23] reported Barrett's mucosa after esophagogastrostomy. The association of Barrett's esophagus with adenocarcinoma of the esophagus has been reported with increasing frequency.[5,6,22,36,39,45] NAEF et al[39] found an 8.5% incidence of esophageal adenocarcinoma in 140 patients with columnar epithelium-lined lower esophagus, and RADIGAN et al[45] reported a 26.3% incidence of esophageal adenocarcinoma in 19 patients with Barrett's esophagus.

The present study represents an effort to experimentally reproduce the columnar epithelium-lined esophagus and consists of a detailed pathological study on esophageal mucosal regeneration. The pattern of epithelial renewal and the nuclear DNA content in Barrett's epithelium were also studied.

Meterials and Methods

Thirty-eight healthy adult mongrel dogs weighing 5 to 21 kg were used.

1. Operative procedure

Fasting animals were operated on under general anesthesia with sodium pentobarbital (20–30 mg/kg of body weight) and controlled respiration. A left thoracotomy through the seventh intercostal space was the standard incision. The esophageal hiatus ring was incised and the stomach was mobilized. Then, an anterior longitudinal incision was made through the wall of the lower 3 cm of the esophagus and the proximal 2 cm of the stomach, thus destroying the lower esophageal sphincter. A semicircle distal esophageal mucosa was removed by dissecting it away from the muscular wall of the distal 4 to 5 cm of the esophagus and esophagogastric

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junction. A transverse two-layer closure of the incision was made as in a WENDEL cardioplasty. The diaphragmatic hiatus was sutured to the gastric fundus, making a hiatal hernia 2 to 3 cm long. The vagus nerves were preserved. This operation is the modification of BREMNER's procedure[11] (Fig. 1).

Antibiotics were used immediately after operation and on the first postoperative day, when oral intake of food and water also resumed. Two weeks after operation, 1% oily histamine, 2 mg/kg of body weight,[28,54] was given to eight of the dogs intramuscularly 3 times a week to stimulate the gastric secretion. However, all of the eight dogs died due to the side effects of the histamine,[24,62] aspiration pneumonia and/or dehydration. Although histamine dosage was decreased from 2 mg/kg to 1 and 0.5 mg/kg, the same results occurred. Therefore, it was concluded that this drug was not useful to this chronic experiment.

The animals which survived the operation were sacrificed 4 to 15 months after operation.

2. Examination

Before operation, all animals were examined as control by esophageal manometry, measurement of esophageal pH, and gastroesophageal reflux test. After operation, all animals were examined repeatedly by esophageal manometry, measurement of esophageal pH, gastroesophageal reflux test, cinefluoroscopy, and esophagoscopy. These examinations were performed under the same general anesthesia as stated above, but the dosage of sodium pentobarbital was decreased.

1) Esophageal manometry[15,40,44,46,47]

Thirty minutes after induction of general anesthesia when the anesthetic depth became constant, esophageal manometry was performed with an open-tipped catheter[44,46] with seven lumens (Argyle Arndorfer-McSteen Esophageal Motility Tube, U.S.A.) and withdrawal curves were graphed. The catheter was connected to a pressure transducer (LPU-0.1, Nihon Koden Kogyo Co., Ltd., Tokyo, Japan), a multi-purpose polygraph (RP-45, Nihon Koden Kogyo Co., Ltd., Japan) and a carrier amplifier (RP-5, Nihon Koden Kogyo Co., Ltd., Japan). During esophageal manometry, bubble-free distilled water was constantly infused at a flow rate of 40 ml/hr through each catheter. Esophageal manometry curves were obtained by continuous

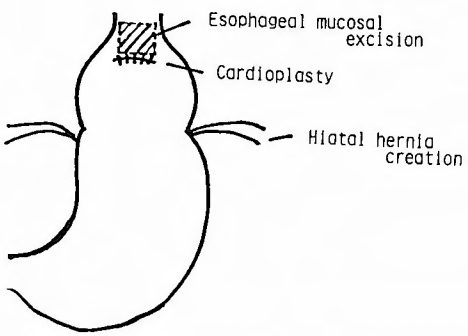

Fig. 1. Esophageal mucosal excision, hiatal hernia creation and WENDEL cardioplasty were performed.

rapid pull-through.[15] Lower esophageal sphincter (LES) pressure was determined in reference to intraesophageal pressure recorded in the intrathoracic esophagus which arbitrarily was considered to be zero.

The hormonal response of the LES was assessed using tetragastrin (Tetragastrin®, Teikoku Zohki Co., Ltd., Japan). A 5 μg/kg dose was administered intravenously and resting pressure was measured 30 minutes later. The length of the LES and the difference between gastric fundic pressure and intrathoracic esophageal pressure were also measured.

2) Esophageal pH measurement

The apparatus consists of a glass-calomel electrode system (GK-282 C, Radiometer, Copenhagen) and a pH meter (PHM 84, Radiometer, Copenhagen) connected to a recorder (Unicorder U-228-2P-129, Nippon Densi Kogaku Co., Ltd., Japan). Firstly, esophagogastric junction was determined by esophageal manometry and esophagoscopy. Secondly, pH electrode was slowly inserted via the mouth into the stomach measuring the esophageal pH. Thirdly, gastroesophageal reflux test was made as below:

3) Gastroesophageal reflux test[29,30,58]

A modification of the method described by TUTTLE and GROSSMAN[58] was used. The pH of the esophagus was monitored with the animal in a supine position. After loading the stomach with 200 ml of 0.1 N HCl, the assembly was placed in such a way as to position the pH electrode 5 cm above the esophagogastric junction previously located. The pH of the esophagus then was raised to above 6.0 by flushing the catheter with water. Gastroesophageal reflux was considered to be present if the pH fell below 3. If reflux was not demonstrated within 3 minutes (free reflux), a series of maneuvers was performed to induce reflux. These consisted of epigastric compression and placing in the Trendelenburg's position. The test was graded as follows:

0, no gastroesophageal reflux
1+, reflux demonstrated with maneuvers
2+, free reflux demonstrated

3. Histological study

The specimens of the lower esophagus and the proximal stomach obtained from each dog were fixed in formalin, embedded in paraffin, serially sectioned 4 μ thick, and stained with hematoxylin and eosin. Then histological studies were performed.

The histological type of Barrett's epithelium, as defined by PAULL et al,[41] was identified for each specimen and the length of regenerated columnar epithelium was measured. The three types of Barrett's epithelium represent the specialized columnar type (S-type), which is characterized by an intestinal-like mucosal appearance with numerous goblet cells; the junctional type (J-type), which has gastric type pits and cardiac mucous glands devoid of parietal cells; and the gastric fundic type (G-type), which has gastric type pits and glands which do contain parietal cells and chief cells.

The inflammation present in each specimen was also graded according to the criteria listed in Table 1. The criteria for inflammation listed under "columnar epithelium" were applied to both esophageal columnar epithelium and gastric epithelium. In addition, the columnar

Table 1. Criteria for grading the degree of inflammatory changes.[14]

Degree of Inflammation	Columnar Epithelium	Squamous Epithelium
0	Moderate no. of mononuclear cells; no polymorphonuclear cells.	Normal rete pegs; normal basal layer; no polymorphonuclear cells.
1+	Increased no. of mononuclear cells; occasional polymorphonuclear cells, but no epithelial transmigration.	Slight increase in rete peg length & thickness of basal layer; no polymorphonuclear cells.
2+	Increased no. of mononuclear cells; moderate no. of polymorphonuclear cells, with occasional epithelial transmigration.	Moderate increase in rete peg length & thickness of basal layer; rare polymorphonuclear cells in epithelium or submucosa (or both).
3+	Increased no. of mononuclear cells; numerous polymorphonuclear cells, with epithelial transmigration with or without pit or gland abscess formation (or both).	Marked increase in rete peg length & thickness of basal layer; numerous polymorphonuclear cells in epithelium or submucosa (or both).

epithelium and the esophageal squamous epithelium were evaluated for dysplasia or atypia.

4. In vitro autoradiography[48]

In the dogs with biopsy-proven Barrett's esophagus, biopsy specimens were taken from the lower esophageal mucosa, Barrett's epithelium and fundic mucosa as control either through esophagoscopy or by esophagotomy under visual control. A part of the specimens was used for labeling experiment and the remainder for routine histological study.

The specimens were brought immediately to our laboratory in a cold phosphate buffer solution (NaCl 8.0 g, KCl 2.0 g, Na_2HPO_4 1.15 g ($Na_2HPO_4 \cdot 12H_2O$ 2.899 g), KH_2PO_4 0.2 g ad aq 1000 ml, pH 7.4). Tissue fragments of 1 mm^3 were placed in 2 ml of pre-warmed medium in a 3 ml vial with a stopper. The medium was prepared from a thymidine-free modification of Ham's F 12 (Nissui Seiyaku Co., Ltd., Tokyo, Japan) supplemented with 20% calf serum. Methyl-tritiated thymidine (TRA 310, specific activity 2 Ci/mmol, The Radiochemical Center, Amersham, England) was added to the final concentration of 5 μCi/ml. A needle was inserted into the vial through a silicone stopper and 3 times the atmospheric pressure of carbogen (oxygen containing 5% CO_2) was applied by connecting to the gas cylinder with a vinyl tube of a small caliber. The rationale for incubation under 3 times the atmospheric pressure of carbogen is to increase availability of oxygen in the depth of tissue, thus improving the labeling pattern.[18,48,49] The vial was shaken at 60 strokes/min for 1 hr in a water bath at 37°C.

After incubation, tissue specimens were fixed in 10% formalin, embedded in paraffin, and sectioned at 4 μ in thickness. The deparaffinized sections were coated with emulsion Type ET 2F (Fuji Photo Film Co., Ashigara) at 40°C. The coated sections were placed in a light-resistant box with a desiccant, exposed for 2–3 weeks at 4°C, and developed in Fuji Rendol (Fuji Photo Film Co.). The autoradiographs were then immediately stained with hematoxylin and eosin, dehydrated, cleared in xylol, and mounted in balsam.

To measure epithelial proliferation, thirty well oriented halves of the pits which contained labeled cells were selected, because the whole gastric pit was not always oriented. The cells were

considered labeled if they had three or more grains over the nucleus. In each half of the pit, the total number of cells (A), the number of cells in the proliferative zone (B) and the number of labeled cells (C) were counted. The proliferative zone which is the region of active DNA synthesis and cell division was defined as the total number of cells below the highest labeled cells on either side of the pit. Then, the labeling index of the pit (C/A), the proportion of the pit which was occupied by the proliferative zone (B/A) and the labeling index of the proliferative zone (C/B) were calculated. Prior animal studies by other investigators have demonstrated agreement between the results obtained by in vivo and in vitro labeling methods.[48,49,59]

5. Microspectrophotometric study of DNA content

The nuclear DNA content was measured in the cells of Barrett's epithelium by Feulgen microspectrophotometry. The measurement of the DNA content was performed upon 4 μ thick paraffin sections from formalin fixed tissue, thus permitting accurate morphological identification of the cells whose nuclei were measured.

After washing the deparaffinized sections, hydrolysis was performed in 5 N HCl for 60 minutes at 25°C ($\pm$3°C), by the method previously reported by TOMONAGA et al.[55,56] After washing, hydrolyzed cells were stained with Schiff reagent for 75 minutes and then bleached in 3 changes of a sulfur dioxide-containing solution for 2 minutes each. Control Feulgen stains were run in parallel. Then, all the sections were dehydrated, cleared in xylol, and mounted in balsam.

The DNA content was measured using a Scanning Micro Densitometer (M85, Nikon Vickers). In each section small mature lymphocytes were measured to give a reference value of DNA; since these cells neither synthesize DNA nor undergo mitosis in the tissue they give a DNA value corresponding to a diploid number of chromosomes (2c). At least 50 lymphocytes were measured in each section. In each section of Barrett's epitheliums diagnosed histologically, 200 to 500 epithelial nuclei were measured. As the control, the normal gastric fundic epithelial cells from 5 dogs and 3 men were used. The DNA content in the cells of adenocarcinoma was also measured in 3 patients with adenocarcinoma of the esophagogastric region. In each section of the control and adenocarcinomas 200 to 500 epithelial nuclei were measured.

6. Statistical analysis

Statistical analysis utilized were Student's t test and chi-square test.[3,19] The data values were expressed as mean values (95% confidence interval).

Results

Of thirty eight dogs operated on, thirty survived the operation. However, twenty two dogs died within 2 months after operation. Twenty five dogs which survived over 2 weeks after operation were examined.

1. Manometric findings

The results are summarized in Table 2. Normal values of dog's LES function were obtained

Table 2. LES evaluation following operation compared to normal control (preoperative) dogs

	Preoperation		Postoperation	
	at Resting	after tetragastrin	at Resting	after tetragastrin
LES Pressure (cm H_2O)	31.31 (28.55-34.07)	51.13 (45.61-56.65)	11.73 (4.34-19.12)	10.05 (2.77-17.33)
G-E Pressure Difference (cm H_2O)	3.21 (2.04- 4.38)	7.14 (4.17-10.11)	0.93 (0- 2.07)	2.25 (0.03- 4.47)
LES Length (cm)	2.81 (2.29- 3.33)	3.61 (2.92- 4.30)	2.12 (0.77- 3.47)	1.90 (0.50- 3.30)

p-Table

		Preoperation-after tetragastrin	Preoperation-at Resting	Preoperation-after tetragastrin	Postoperation-at Resting
LES Pressure	Preoperation-after tetragastrin		***		
	Postoperation-at Resting		***	***	
	Postoperation-after tetragastrin		***	***	N. S.
G-E Pressure Difference	Preoperation-after tetragastrin		**		
	Postoperation-at Resting		**	***	
	Postoperation-after tetragastrin		N. S.	**	N. S.
LES Length	Preoperation-after tetragastrin		*		
	Postoperation-at Resting		N. S.	**	
	Postoperation-after tetragastrin		N. S.	**	N. S.

Results expressed as mean values (95% confidence interval)
LES. lower esophageal sphincter
G-E Pressure Difference: the difference between gastric fundic pressure and intrathoracic esophageal pressure
***: $p<0.01$; **: $p<0.05$; *: $p<0.10$
N.S.: not significant

from a preoperative manometric study as control. In the control group, the mean resting LES pressure was 31.31 cmH_2O (28.50–34.07) and the mean gastrin-stimulated LES pressure was 51.13 cmH_2O (45.61–56.65). This response, 171.48% (143.25–199.71) of the resting LES pressure, was significant ($p<0.01$). The mean difference between gastric fundic pressure and intrathoracic esophageal pressure was 3.21 cmH_2O (2.04–4.38) at resting and parenterally administered tetragastrin produced a significant increase in the difference between gastric and esophageal pressure ($p<0.05$). The mean length of the LES was 2.81 cm (2.29–3.33) and was significantly increased in response to gastrin stimulation compared to at resting ($p<0.10$).

After operation, the LES pressure and the difference between the pressure of gastric fundus and intrathoracic esophagus were significantly decreased compared to the controls ($p<0.01$, $p<0.05$). Furthermore, the LES pressure was not found in some of the postoperative subjects. The length of the LES at resting was unchanged by the operation. While the LES responded to tetragastrin well in the controls as shown above, no response was observed after operation (Fig. 2). These results indicate that the LES function of the dogs operated on were completely destroyed on the basis of manometric study.

2. Results of esophageal pH measurement and gastroesophageal reflux test

In all of the control dogs, the esophageal pH was above 6.8 and the gastric pH was below 2.0. The gastroesophageal pH curve showed clearly a reversal point in the controls at the esophagogastric junction. Gastroesophageal reflux test showed no reflux in any of the control dogs.

Gastroesophageal reflux test after operation showed 2+ in 22 dogs and 1+ in 3 dogs. Significant gastroesophageal reflux was demonstrated after operation.

3. Cinefluoroscopic findings

Upper gastrointestinal radiography with the stomach loaded with barium demonstrated hiatal hernia and severe reflux of barium into the esophagus in all the dogs examined after operation (Fig. 3).

4. Esophagoscopic findings

Healing of the experimental ulcer occurred from the sides of the esophagus and the stomach. Two months after operation, there was columnar re-epithelization a few millimeters long upward to the ulcer. Three months after operation, the columnar re-epithelization of the ulcer was extended slightly more. Different degrees of columnar re-epithelization were seen in individual dogs. Over four months after operation there were remarkable columnar re-epithelizations in

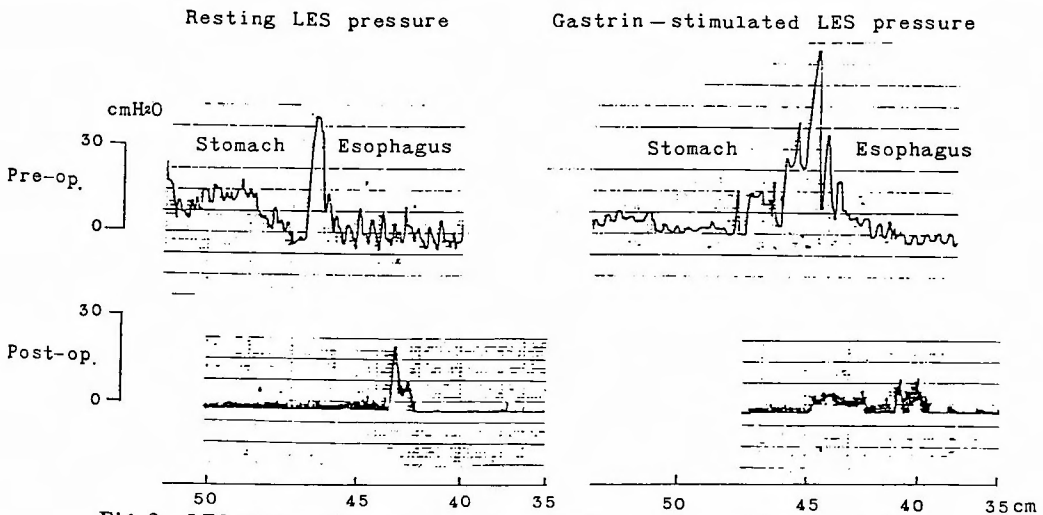

Fig. 2. LES pressure after operation compared with normal control (preoperative) dog

Fig. 3. X-ray film of dog No. 1 showing hiatal hernia and severe gastroesophageal reflux

all the dogs which survived, apparently confirming the diagnosis of the experimental Barrett's esophagus. Gastroesophageal reflux was remarkable but esophagitis was mild even around the ulcer.

5. Histological findings

Barrett's esophagus was identified in 2 of 22 dogs which died within 2 months after operation. Of eight dogs which survived more than two months after operation, seven dogs had regenerated columnar epithelium of the lower esophagus. These nine dogs were used in the histological

Table 3. Regenerated columnar (Barrett's) epithelium after mucosal excision in the dogs

Dog No.	Days	Length of Barrett's Epithelium (mm)			Inflammation			Gastro-esophageal reflux test	Postoperative LES pressure (cm H_2O)
		G-type	J-type	Total	Barrett's mucosa	Gastric Fundus	Esophagus		
1	442	16	—	16	1+	0	1+	2+	7.5
6	20	2	—	2	0	0	1+	1+	12
7	56	2.4	4	6.4	1+	0	1+	2+	6
8	377	15.2	—	15.2	1+	0	1+	1+	21
9	149	9.8	5.1	14.9	1+	0	1+	2+	7.5
13	179	4.3	0.5	4.8	2+	1+	2+	2+	10.5
28	144	7.2	19.5	26.7	0	0	2+	2+	3
29	96	-	15.6	15.6	1+	0	1+	2+	4.5
37	240	6.9	18	24.9	1+	1+	2+	2+	8.5

study (Table 3).

The regenerated columnar (Barrett's) epitheliums were 2 to 26.7 mm long, with a mean of 14.06 mm (11.23–16.89). The relation between the length of Barrett's epithelium and the degree of acid gastroesophageal reflux or esophageal manometric findings could not be determined

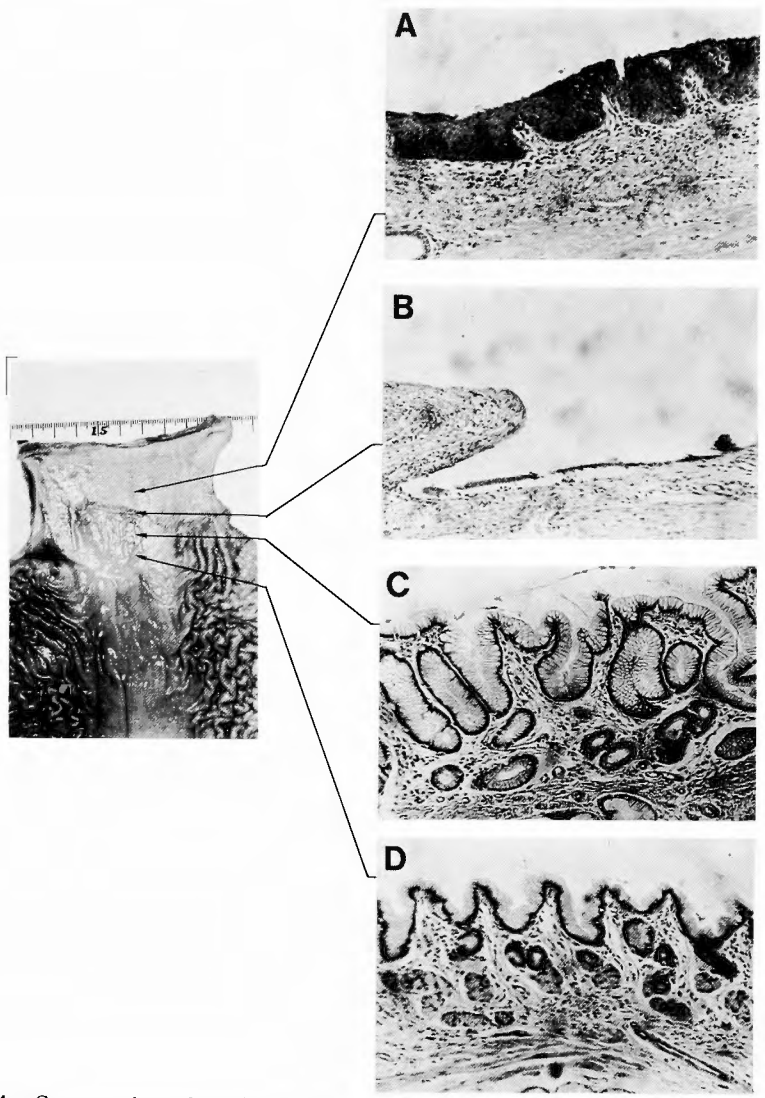

Fig. 4. Gross specimen from dog No. 28 144 days after operation. Histologic appearance of mucosal lining at various level is indicated. Denuded area has been covered by columnar epithelium.

A. Basal cell hyperplasia, location of papillae close to the epithelial surface[29,30] and fibrosis of the submucosal layer are shown at the distal esophagus. ×100

B. At the regenerated edge, a single layer of cuboidal or flat epithelium without formation of gastric pits was observed. ×100

C. Gastric pits and cardiac glands are shown (the junctional type of Barrett's epithelium). ×100

D. Gastric pits, parietal cells and chief cells are shown (the gastric fundic type of Barrett's epithelium). ×100

because the interval after operation varied greatly among dogs.

The specimens from the nine dogs which had Barrett's epithelium were the G-type in 3, the J-type in 1, and both the G-type and the J-type in 5 (Fig. 4). No S-type was found. In these dogs which had two types of Barrett's epithelium, the J-type epithelium was always located proximal to the G-type epithelium. At the regenerated edge, 4 specimens had a single layer of cuboidal or flat epithelium without formation of gastric pits. The J-type and the G-type epithelium which were thought to be spreading from the stomach upward to the ulcer resembled the normal cardiac or fundic mucosa histologically. On the other hand, the esophageal ulcer was re-epithelized by squamous epithelium from above. The new squamous epithelium was only one or two layer thick at the regenerated edge but several layers thick at more proximal levels.

All specimens were negative for severe atypia, dysplasia, or neoplasms. In two cases (No. 8, 29), hyperplasia with papillary growth and hyperchromatism was seen in the regenerated columnar epithelium (Fig. 5).

The degree of inflammation in the specimens was as a whole mild. On a scale of 0 to 3+, the mean of Barrett's epitheliums was 0.89 (0.69–1.09), the mean of the fundic mucosa 0.22 (0.07–0.37), the mean of the squamous epitheliums 1.33 (1.16–1.50) (Table 3). Chronic esophagitis with fibrosis of the submucosal layer was diagnosed in 5 cases.

6. Autoradiographic findings

Five dogs (No. 1, 9, 8, 28, 37) diagnosed as having Barrett's esophagus were used in the study. The specimens from the five dogs included 5 control gastric fundic mucosas, 3 J-type Barrett's epitheliums and 5 G-type Barrett's epitheliums.

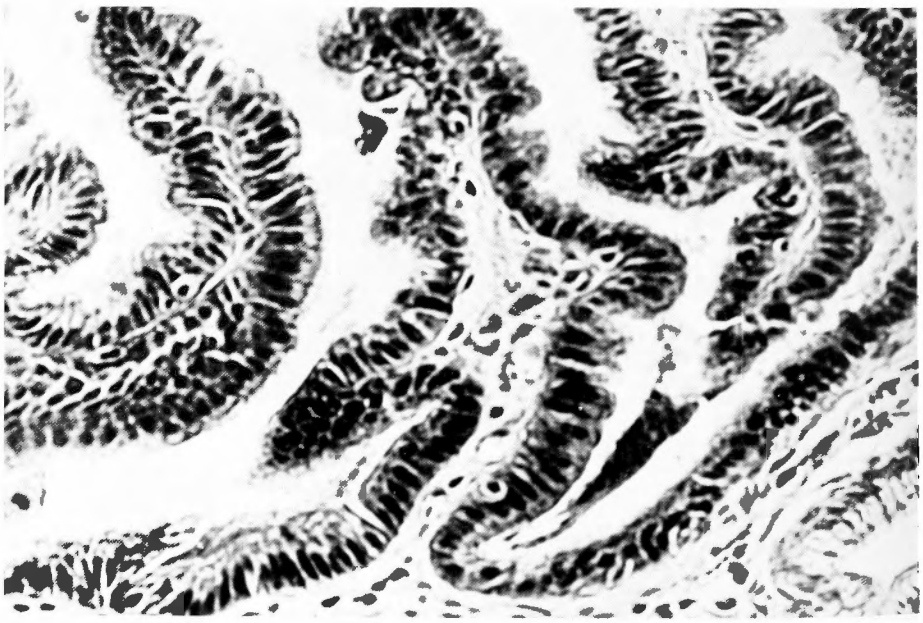

Fig. 5. Hyperplasia is seen with papillary growth and hyperchromatism in the Barrett's epithelium of dog No. 8. ×200

In half of the pit, the number of cells in the proliferative zone (B) and the number of labeled cells (C) were significantly greater in the G-type and the J-type epithelium than in the control epithelium ($p<0.01$). The total number of cells per half of the pit (A) was significantly greater only in the G-type epithelium than in the control epithelium ($p<0.01$). The labeling index of the pit (C/A) was significantly greater in the J-type and the G-type epithelium than in the control epithelium ($p<0.05$, $p<0.10$). The mean labeling indices of pits were 9.18% (7.38–10.98) in the J-type, 8.15% (7.28–9.02) in the G-type, and 7.18% (6.48–8.58) in the control. The labeling index of the squamous epithelium of the esophagus was 9.03% (7.15–10.91). The proportion of the pit which was occupied by the proliferative zone (B/A) was significantly greater in the G-type and the J-type epithelium than in the control epithelium ($p<0.01$, $p<0.01$). The mean B/A were 38.96% (38.30–42.62) in the G-type, 38.73% (33.32–44.14) in the J-type and 28.55% (25.80–31.30) in the control. These findings indicated an abnormal epithelial renewal in

Table 4. Autoradiographic findings in Barrett's epithelium compared to the control gastric fundic epithelium

	Barrett's Epithelium		Control Gastric Fundic Epithelium
	G-type	J-type	
A	35.47 (35.15-35.79)	32.10 (27.81-36.39)	29.59 (27.79-31.39)
B	14.20 (13.99-14.41)	12.27 (9.64-14.90)	8.39 (7.43- 9.35)
C	2.85 (2.42- 3.28)	2.76 (2.24- 3.28)	1.97 (1.79- 2.15)
C/A (%)	8.15 (7.28- 9.02)	9.18 (7.38-10.98)	7.18 (6.48- 8.58)
B/A (%)	38.96 (35.30-42.62)	38.73 (33.32-44.14)	28.55 (25.80-31.30)
C/B (%)	23.44 (20.88-26.00)	26.24 (21.98-30.50)	30.53 (27.19-33.87)

p-Table

J-type	N.S.		N.S.		N.S.	
Control	***	N.S.	***	***	***	***
	G-type	J-type	G-type	J-type	G-type	J-type
	A		B		C	
J-type	N.S.		N.S.		N.S.	
Control	*	**	***	***	***	N.S.
	G-type	J-type	G-type	J-type	G-type	J-type
	C/A		B/A		C/B	

A : Total number of cells per a half of pit
B : Number of cells in the proliferative zone
C : Number of labeled cells within the proliferative zone
C/A : Labeling index of the pit
B/A : Proportion of the pit which is occupied by the proliferative zone
C/B : Labeling index of the proliferative zone
***: $p<0.01$; **: $p<0.05$; *: $p<0.10$
N.S.: not significant

Barrett's epithelium, that is, expansion of the proliferative zone beyond its normal limits. The labeling index of the proliferative zone (C/B) was significantly greater only in the control epithelium, 30.53% (27.19–33.87) than in the G-type epithelium, 23.44% (20.88–26.00) ($p<0.01$). The mean labeling index of the proliferative zone was 26.24% (21.98–30.50) in the J-type epithelium. Between the two types of Barrett's epithelium, no significant differences were found in C/A, B/A and C/B (Table 4).

In the control fundic mucosa, labeled cells were seen in the lower one-third of the gastric pits and labeled surface cells were not found. In Barrett's epithelium, extension of the proliferative zone toward the upper part of the pit was recognized, and three G-type and one J-type epitheliums had labeled surface cells (Fig. 6). No labeled cells were seen in the cardiac glands and the proper esophageal glands, suggesting that these glands had no proliferative cells. The cuboidal or flat epithelium at the regenerated edge had no labeled cells, either. The mean labeling index of the hyperplastic columnar epithelium with papillary growth which was seen in two cases of Barrett's esophagus was 6.18%.

7. Microspectrophotometric findings of DNA content

Six dogs with Barrett's epithelium were used in the study. The sections included 5 G-type epitheliums and 4 J-type epitheliums.

Most nuclei of the control gastric fundic epithelial cells from dogs and men showed DNA values between 2c and 4c, and "peak" values of DNA between 2c and 2.5c. The frequency of the DNA values above the 4c level was less than 5%. This is what might be expected in normal

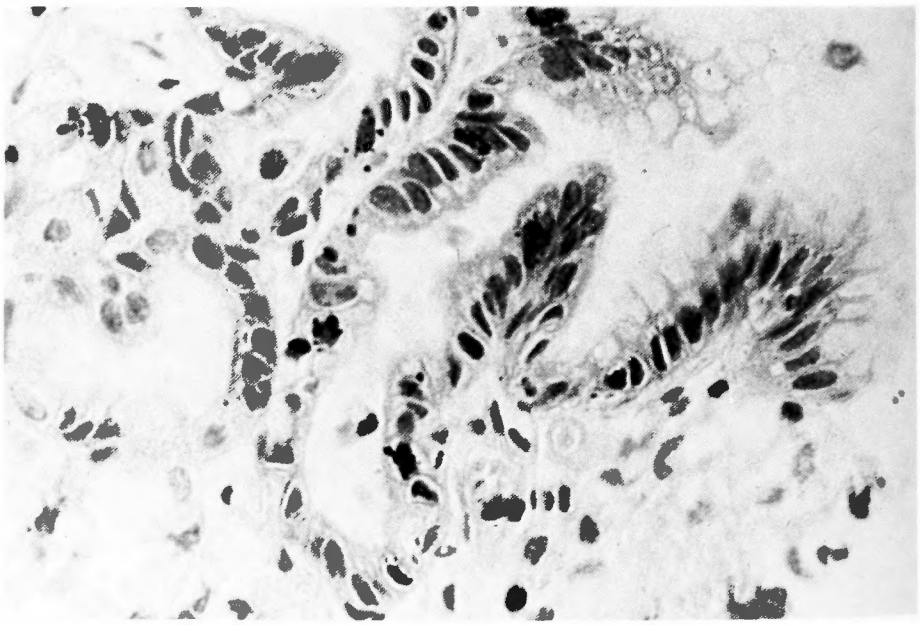

Fig. 6. In vitro autoradiography.
The gastric fundic type of Barrett's epithelium of dog No. 1 is shown. Labeled cells are seen in the upper part of the pit. ×400

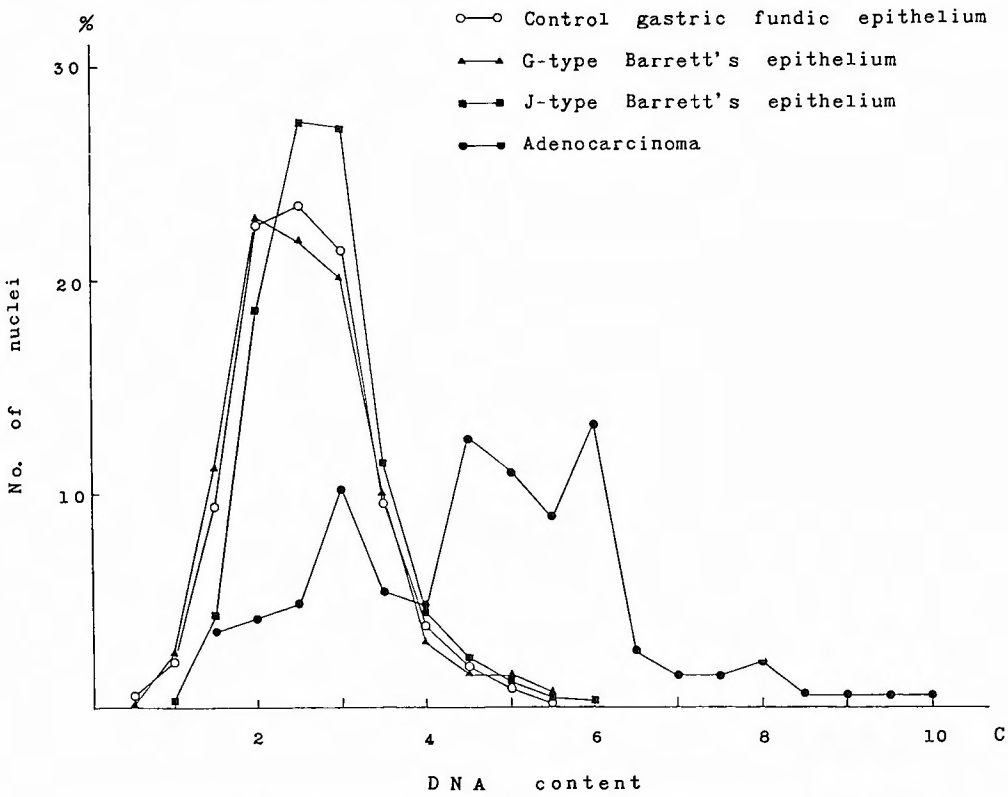

Fig. 7. DNA values of the epithelial cells in the normal gastric fundus, Barrett's mucosa and adenocarcinoma

proliferation of benign tissue.

The specimens histologically diagnosed as the G-type or the J-type Barrett's epithelium showed almost the same findings, indicating the benign nature of these lesions in the specimens. However, two (No. 9, 37) of the G-type epitheliums and two (No. 9, 29) of the J-type epitheliums showed DNA values above the 4c level at the frequency of 5 to 10%. No significant differences were found between Barrett's epithelial cells and the control (Fig. 7).

In the invasive adenocarcinomas of men a variable proportion of nuclei gave DNA values above the level of 4c corresponding to an aneuploid number of chromosomes. The proportion of such cells varied in the three carcinomas measured. This result was expected from previous work on carcinomas of other human tissues and confirmed previous findings, apart from a greater spread of values obtained which was due in part to measuring interphase nuclei.

Report of cases

Case 1. A 62-year-old man had a 3-year history of dysphagia. A month before admission, he could swallow only liquids. He complained of a recent 7-kg weight loss. He was a heavy drinker and a heavy smoker. Upper gastrointestinal examination showed a sliding hiatal hernia and an ulcerated stricture of the lower esophagus. A partial esophagogastrectomy and eso-

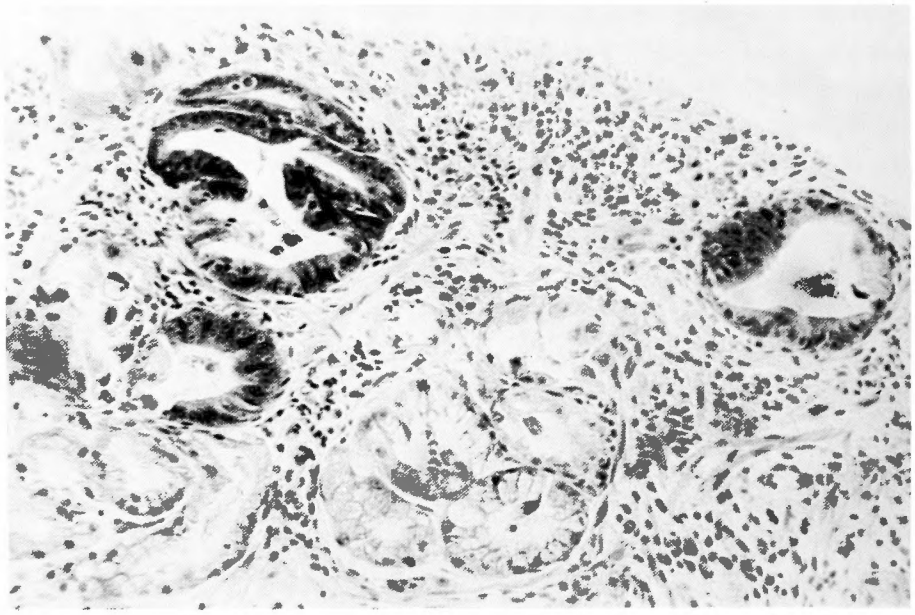

Fig. 8. A part of the columnar epithelium showed severe dysplasia (Case 1). ×100

phagogastrostomy were performed in July 1970. Resected specimen demonstrated a columnar epithelium-lined lower esophagus with an esophageal ulcer. The columnar epithelium was histologically diagnosed as the J-type Barrett's esophagus. A part of the columnar epithelium showed severe dysplasia (Fig. 8). Examination of the resected specimen demonstrated no malignancy.

Case 2. A 71-year-old woman had a 10-year history of heartburn. Two weeks before admission, she developed dysphagia. Upper gastrointestinal radiography revealed a sliding hiatal hernia and a mass lesion at the esophagogastric junction. A partial esophagogastrectomy and esophagogastrostomy were performed in July 1979. Examination of the resected specimen demonstrated a squamocolumnar junction just above the proximal margin of the tumor. The neoplasma was a poorly differentiated adenocarcinoma (Fig. 9). The columnar epithelium of the lower esophagus was the J-type Barrett's epithelium without parietal cells and showed moderate dysplasia. It was thought that the adenocarcinoma arose from Barrett's epithelium. The patient died due to recurrence of the carcinoma about nine months after operation.

Case 3. A 78-year-old woman had severe anemia. There were no episodes of dysphagia and heartburn. Upper gastrointestinal examination showed a sliding hiatal hernia and a constricting lesion at the midesophagus (Fig. 10). Esophagoscopy showed a short segment of ulcerated stricture at a 23-cm level from the incisors. The esophagogastric junction was found at a 34-cm level from the incisors. Biopsy specimens taken from the esophagus at the stricture showed columnar mucosa with numerous goblet cells and villi, which confirmed the diagnosis of the S-type Barrett's esophagus (Fig. 11). Malignancy was not demonstrated in Barrett's epithelium. Surgical treatment was not performed and she is being followed up.

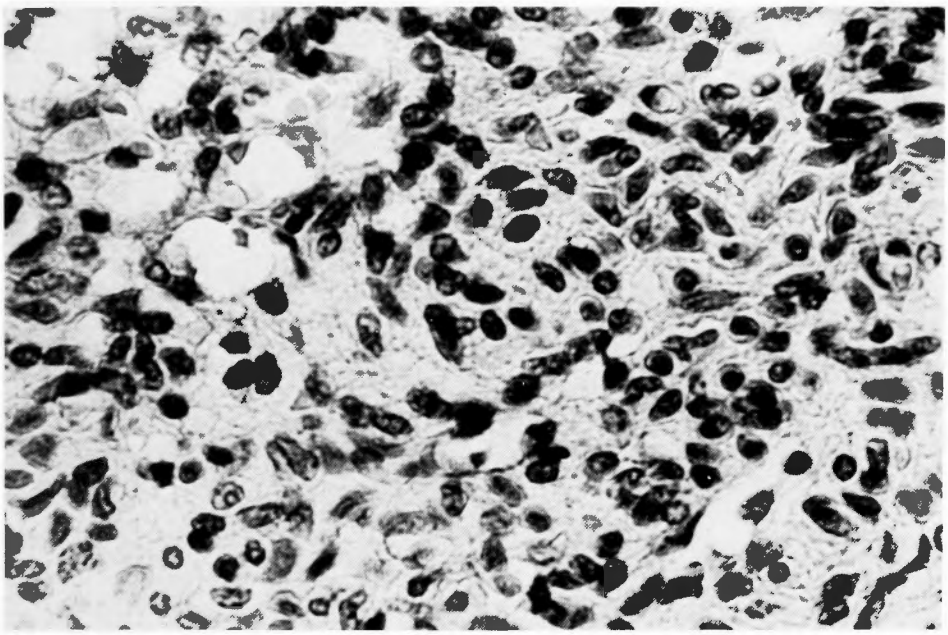

Fig. 9. Poorly differentiated adenocarcinoma (Case 2) × 200

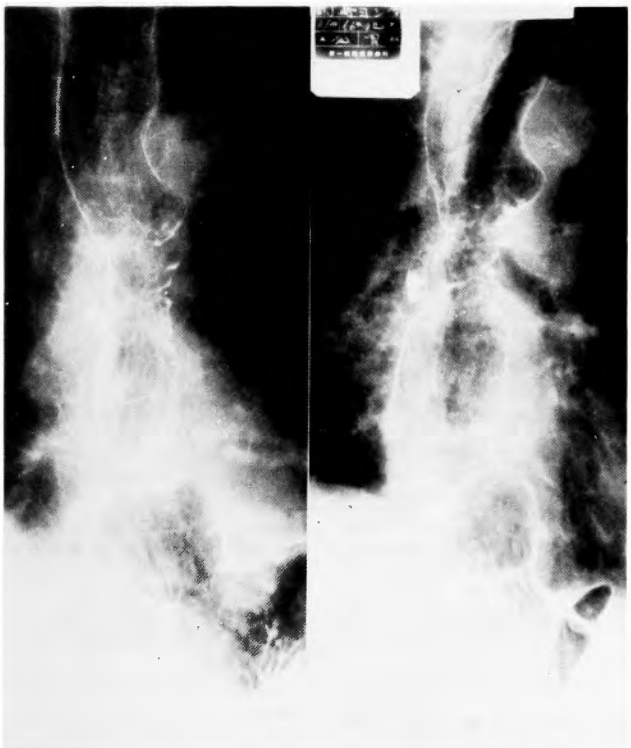

Fig. 10. Upper gastrointestinal examination of Case 3 shows a sliding hiatal hernia and a constricting lesion at the midesophagus.

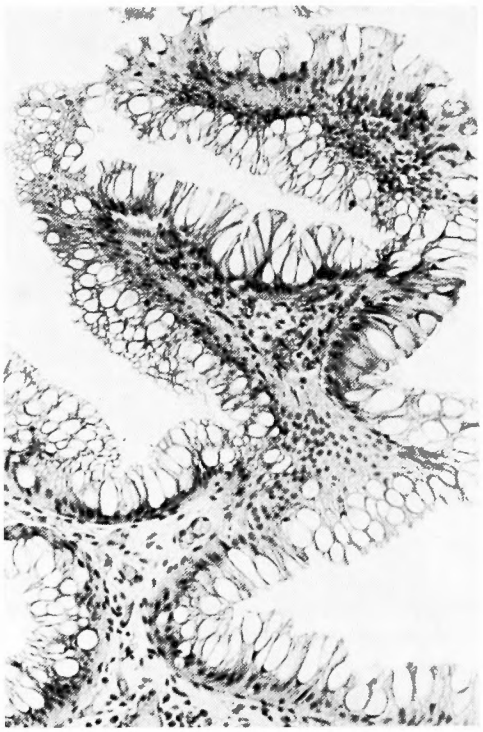

Fig. 11. The specialized columnar type of Barrett's epithelium with numerous goblet cells and villi is seen in Case 2. ×100

Case 4. A 58-year-old man was admitted with a one-year history of regurgitation and heartburn. He had drunk heavily for thirty years. Upper gastrointestinal examination showed a sliding hiatal hernia and a stricture at the lower esophagus. Esophagoscopy showed an ulcerated stricture with a nodular protrusion just above the esophagogastric junction. A partial esophagogastrectomy and esophagogastrostomy were performed. A resected specimen demonstrated a columnar epithelium-lined lower esophagus with an esophageal ulcer. This epithelium was histologically diagnosed as the J-type Barrett's esophagus. No malignancy was demonstrated.

In these four cases, the DNA contents of the nuclei from the four Barrett's epitheliums and an adenocarcinoma were measured. Proportions of the Barrett's epithelial cells had values in the normal range. The same findings were demonstrated even in the cells with atypia found in Barrett's epitheliums of two of the patients (case 1 and 2). Poorly differentiated adenocarcinoma (case 2) showed frank aneuploidy with a very wide spread of DNA values (Fig. 12).

Discussion

The columnar epithelium-lined (Barrett's) esophagus is associated with esophageal stricture, esophageal ulcer, hiatal hernia, short esophagus, esophagitis and occasionally adenocarcinoma.

About the etiology of Barrett's epithelium, the often-quoted concepts are that this condition

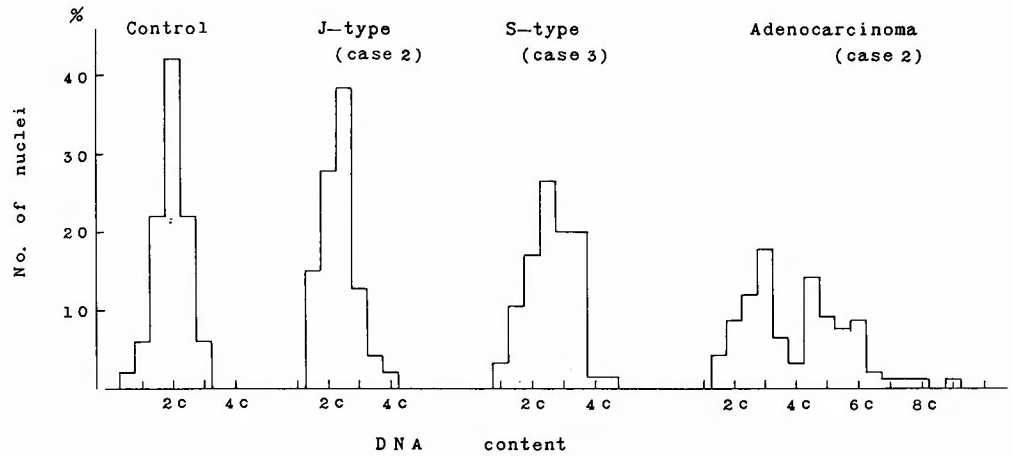

Fig. 12. DNA values of the epithelial cells in the individual clinical cases

is congenital as a result of incomplete replacement of the embryonic columnar epithelium by squamous epithelium,[1,33] or acquired, owing to chronic reflux of gastric contents into the esophagus.[2,11,21,23,57,61] It has also been proposed that examples of both etiologies occur.[25,38,42] The advocates of the congenital theory rightly point out that fetal esophagus is lined with a columnar-type epithelium and that islands of ectopic gastric epithelium may be found in the esophagus of a newborn infant. Excepting these cases, Barrett's esophagus is thought to be an acquired disorder in which the squamous epithelium is destroyed by gastroesophageal reflux and is replaced by columnar epithelium. This experimental study shows that columnar epithelium can cover part of the dog's distal esophagus under the presence of acid gastroesophageal reflux after the esophageal mucosal lining has been surgically removed. This finding supports the acquired theory.

While the relation between the extent of the re-epithelization by columnar epithelium and the degree of acid gastroesophageal reflux was not clarified in this study, BREMNER et al[11] reported that the more excessive acid gastroesophageal reflux was, the more extensive columnar re-epithelization was. The major causative factor of Barrett's esophagus is acid gastroesophageal reflux, but cases of Barrett's esophagus following total gastrectomy[37] and complicating lye ingestion with the sparing of the distal esophagus[53] have been reported. Therefore, further investigation on the causative factors of Barrett's esophagus other than acid reflux is desirable.

Experimental Barrett's epithelium of the dogs were the G-type in 3, the J-type in 1, and both in 5. But the S-type as seen in patients with Barrett's esophagus was not found. This result is reasonable because intestinal metaplasia is not seen in the stomach of dogs. PAULL[41] and HAGGITT[22] reported that when three or more of Barrett's epithelium were concurrently present, the S-type was always the most proximal and the G-type was the most distal epithelium. They further showed that the J-type was interposed between the G-type and the S-type. This experiment also showed that the J-type was always located proximal to the G-type. This arrangement of two types of columnar epithelium is similar to that of normal cardiac and fundic mucosa. Moreover, the regenerated columnar epithelium was in continuity with the lining of the stomach.

These experimental findings support the theory that the mechanism by which the columnar epithelium develops is the spreading of the cardiac or gastric mucous membrane upward into the region of the damaged squamous epithelium.

At the regenerated edge, a single layer of cuboidal or flat epithelium was observed without formation of gastric pits and autoradiographic findings indicated that these epithelial cells had no proliferative activity. As reported by YAMASHITA,[63] this fact seems to deny the concept that new glands are produced by invagination of a single layer epithelium.

There was some controversy as to whether Barrett's epithelium has a secretory capacity or not. Some authors[12,35,57] showed that this epithelium could secrete mucus, acid, pepsin, and gastrin. Paneth cells in Barrett's esophagus were also identified.[52] BREMNER et al[11], on the other hand, reported that parietal cells were not identified in experimental Barrett's epithelium. This experiment, however, showed that Barrett's epithelium had numerous parietal cells. It is thought that the experimental Barrett's epithelium may secrete an acid as normal fundic mucosa does.

Another aspect of the unresolved controversy concerns the question of whether Barrett's esophagus is a "premalignant" lesion or not. The incidence of adenocarcinoma of the esophagus appears to increase in the presence of Barrett's epithelium and the reported incidences of esophageal adenocarcinoma in Barrett's esophagus were 8.5%,[39] 20%,[20] and 26.3%.[45] HAGGITT et al[22] found that, of 14 cases of primary adenocarcinoma, 12 had it in Barrett's epithelium. Moreover, they reported that, in two of their patients, carcinoma developed in the columnar epithelium-lined esophagus three years and three years and a half respectively after antireflux procedure. BRAND et al[10] also reported that in one patient an adenocarcinoma developed four years after an unsuccessful NISSEN repair. NAEF et al[39] reported that an adenocarcinoma of the esophagus developed in Barrett's esophagus two years and a half after cure of active reflux esophagitis by NISSEN fundoplication. One of our 4 clinical cases with Barrett's esophagus was complicated by adenocarcinoma and it showed moderate dysplasia of the columnar epithelium adjacent to the tumor. In another patient the columnar epithelium showed severe dysplasia. These facts suggest that Barrett's epithelium has a malignant potential.

Abnormal patterns of epithelial renewal have been associated with frank neoplasia or preneoplastic lesions elsewhere in the gastrointestinal tract.[7,8,16,60] It is known that in the normal gastric mucosa the proliferative zone is limited to the lower one-third of the gastric pit and the proliferative cells differentiate to the surface epithelium and the glandular cells.[31,32,50] Our autoradiographic study also showed that in the control gastric mucosa the proliferative zone was limited to the lower one-third of the gastric pit and no surface cells was labeled. Previous studies[31,32,43,50] are not necessarily in accord with each other in the values of labeling indices, probably due to differences in materials and methods used by individual investigator.

In this study, autoradiography of experimental Barrett's epithelium showed that the proliferative zone was extended toward the upper part of the pit and the labeling index of the pit was greater compared to the control fundic mucosa. Moreover, some of the dogs with Barrett's

epithelium had labeled surface cells, indicating changes in the size of the proliferative zone.[34,36] These findings indicate the increased epithelial proliferation in Barrett's epithelium. Two of the dogs with Barrett's epithelium had hyperplastic lesions with papillary growth and nuclear hyperchromatism. The results of this study suggest that Barrett's epithelium may have a close association with a premalignant lesion. Mild inflammation was observed in Barrett's mucosa. The relationship between an inflammatory condition and enhanced epithelial proliferation has been documented for other gastrointestinal tissues.[7,14,16,43] The theory[14] that atrophic gastritis may be a premalignant status is interesting. The labeling index of the proliferative zone in the control fundic mucosa was significantly greater than in the G-type Barrett's epithelium but the cause of it could not be clarified. Probably, more cells in the expanded proliferative zone of the G-type Barrett's epithelium may be out of the S-phase—that is, the duration out of the S-phase may be prolonged or the number of the cells in the G_0-phase may be increased in this situation. PELLISH[43] reported that the pattern of epithelial proliferation in Barrett's epithelium is general was similar to that found in other gastrointestinal columnar epithelia but a minority of patients with Barrett's epithelium might have an expanded proliferative zone. HERBST[26] also reported that labeling of surface columnar cells after brief exposure to ^{3}H-thymidine was present in Barrett's epithelium. Both reports suggested close relationship between Barrett's epithelium and its malignancy.

It is known that DNA content in tumor nuclei is usually increased compared to benign tissue which has DNA values between 2c and 4c.[9,17,27,51] It seems reasonable that the presence of aneuploid DNA values is at least an indication of potentially malignant neoplastic growth. HIROSE[27] and SASAKI[51] reported that DNA measurement might be of diagnostic help in the borderline lesions between malignant and benign changes of the stomach. This microspectrophotometric study of DNA content shows that the cells of the control epitheliums have DNA values between 2c and 4c, and that the cells of invasive carcinomas have largely aneuploid values of DNA. These findings are consistent with the results of the previous studies by other investigators. The cells of Barrett's epithelium had DNA values in the normal ranges. Although a part of Barrett's epitheliums seemed to show a slight shift to a higher average DNA content, no significant differences were found. On the basis of DNA values there was no evidence that Barrett's epithelium had a malignant potential.

There probably is an increased incidence of adenocarcinoma occurring in the columnar mucosa in Barrett's esophagus. Initial reports indicated that the columnar epithelium which lines the distal esophagus does not necessarily revert to normal following antireflux operations.[35,39] More recently, reversion to normal squamous tissue has been reported.[10,45] Accordingly, the finding of esophageal columnar epithelium in patients with reflux esophagitis should be considered a strong indication for an antireflux operation and continued follow-up after treatment is important in all cases. If carcinoma develops in such cases, resective surgery should replace present-day non-resective antireflux operations.

Conclusion

1. Barrett's esophagus was experimentally created under reflux esophagitis, using adult mongrel dogs. It is concluded that Barrett's esophagus is an acquired condition and develops by the spreading of the cardiac or gastric mucous membrane upward into the region of the damaged squamous epithelium.

2. Experimental Barrett's epitheliums had gastric pits, cardiac glands, parietal cells and chief cells but had no villi and goblet cells.

3. Histological findings in experimental Barrett's epithelium showed no atypia but a minority of Barrett's epitheliums showed hyperplastic lesions. A microspectrophotometric study of DNA content in Barrett's epithelium showed DNA values in the normal ranges but a minority of Barrett's epithelium showed a slight shift to a higher average DNA content. These results do not indicate that Barrett's esophagus has a malignant potential.

4. Autoradiographic findings, however, showed that the proliferative zone in the experimental Barrett's epithelium was expanded beyond its normal limit, thus suggesting that Barrett's epithelium is a premalignant condition.

5. One of the four patients with Barrett's esophagus had an adenocarcinoma in the lower esophagus and the columnar epithelium of the patient showed moderate dysplasia. In another patient the columnar epithelium showed severe dysplasia. These findings also suggest that Barrett's epithelium is a premalignant lesion.

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和文抄録

Barrett 食道に関する実験的ならびに臨床的検討

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Columnar epithelium-lined lower esophagus (Barrett 食道) に関して，その成因および合併症（特に発癌性）の問題を明らかにするため，以下の実験を行った．

1) 雑種成犬38匹に，下部食道粘膜切除による人工的食道潰瘍を作成し，同時に食道裂孔ヘルニアの作成と Wendel 噴門形成術を併施したのち，逆流性食道炎発生下における食道潰瘍の治癒過程を観察した．手術前後の検査で，オープン・チップ法，rapid pull-through technique による食道内圧測定では，術前静止圧は 31.31 cmH_2O (28.5～34.07) であり，テトラガストリン刺激時には 51.13 cmH_2O (45.61～56.65) と上昇したが，術後には静止圧は 11.73 cmH_2O (4.34～19.12) と有意に低下し ($p<0.01$)，ガストリンに対する反応は全くみられなくなった．又，胃食道内圧差も，術前に比較して術後は有意に低下していた ($p<0.05$)．食道内 pH 測定，および 0.1N HCl 200 ml を注入したのち，食道胃接合部の 5 cm 口側において食道内 pH を測定する胃食道逆流試験の結果では，術前には全く胃食道逆流がみられなかったのに対して，術後は著明な胃食道逆流が起こっていることが示された．胃食道透視でも，著明な胃食道逆流がみられ，これらの検査によって，術後のイヌでは，下部食道噴門括約機構が完全に破壊されていることが示された．

2) 術後2カ月～1年3カ月間生存した10匹のイヌのうち，9匹に食道潰瘍の部に円柱上皮の再生がみられ，実験的 Barrett 食道が作成されていた．Barrett 上皮の長さは平均 14.06 mm (11.23～16.89) で，Paull の分類によると，胃小窩，壁細胞，主細胞を有する gastric fundic type (G-type) の上皮が3例，胃小窩，噴門腺を有する junctional type (J-type) の上皮が1例，G-tye と J-type の両者を有する上皮が5例であり，杯細胞，絨毛構造を有する specialized columnar type (S-type) の上皮はみられなかった．J-type の上皮は常に G-type の上皮の口側に位置し，正常胃上皮と連続性をもって再生していた．これらの結果から，Barrett 食道は，後天性の変化であり，噴門部又は胃の粘膜の口側への進展によるものであることが示された．

3) 実験的 Barrett 上皮には，組織学的には異型性はみられなかったが，一部の症例では乳頭状増殖と核過染性を伴った過形成の所見がみられた．Barrett 上皮の組織切片を使っての Feulgen 染色による核 DNA 量測定の結果では，正常胃底部上皮と Barrett 上皮の間に有意差は見出されなかったが，^{3}H-thymidine による in vitro Autoradiography における観察では，G-type および J-type の Barrett 上皮は正常胃底部上皮に比較して，胃小窩標識率は高値を示し ($p<0.10$, $p<0.05$)，増殖帯の拡大 ($p<0.01$, $p<0.01$) がみられた．この結果より，Barrett 上皮が前癌病変であることが示唆された．

4) 教室では4例の Barrett 食道を経験し，そのうち1例には Barrett 上皮の部に食道腺癌を合併し，付近の Barrett 上皮に異形成を認めた．他の1例には Barrett 上皮に著明な異形成を認めた．これらの所見によっても，Barrett 上皮が前癌病変であることが示唆された．